bio-protocol Preprint

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Generation of SH-SY5Y HTT knockout cell lines by CRISPR-Cas9

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An abbreviated version of this protocol was published in BMC Medical Genomics in Jul 2021

RNA-seq analysis reveals significant transcriptome changes in huntingtin-null human neuroblastoma cells

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Detailed protocol

1. Rehydrate the multi-guide sgRNA
    i. Briefly centrifuge the tube containing the dried multi-guide sgRNA to ensure the pellet is collected at the bottom.
    ii. Carefully rehydrate the 1.5nmols sgRNA in 15ul nuclease free buffer (1X TE buffer provided by the company). Pulse vortex for 30 seconds to ensure complete mixing. Final concentration is 100uM (100pmol/ul).
    iii. Store at -20C (>20uM stable for up to 3 years if not repeatedly thawed).
2. Dilute the multi-guide sgRNA
    i. Add 6ul 100uM sgRNA to 14ul nuclease -free water to make a total of 20ul 30uM multi-guide sgRNA (30pmol/ul). Use immediately, or store at -20C for up to 3 years if not repeatedly thawed.
3. Transfect the cells (electroporation)
    i. Seed cells: subculture SH-Sy5Y cells 2 days before electroporation so that they are 70-80% confluent on the day of transfection.
    ii. On the day of transfection:
        a. Pre-warm 0.5ml normal growth medium without antibiotics in each well of a 24-well cell culture plate per reaction.
        b. Assemble RNA complexes (9:1 sgRNA:cas9 ratio): per cell line: Add 3ul 30uM sgRNA into a tube followed by 0.5ul cas9, then add 3.5ul resuspension buffer R (total 7ul). Mix and incubate at room temperature for 10min.
        c. Prepare cells: trypsinize cells and count cell numbers. Transfer 2 x 10^6 cells to a microcentrifuge tube, Centrifuge cells and wash cells once with PBS, centrifuge again. Remove PBS and resuspend cells in 50ul resuspension buffer R.
        d. Add 5ul cell suspension to each RNP solution (7ul) to make 12ul of cell-RNP solution per reaction.
        e. Aspirate 10ul cell-RNP solution to a 10ul Neon tip.
        f. Electroporate using the following parameter. 1,100 V, 50 ms, 1 pulse.
        g. After transfection, quickly resuspend the cells in 100ul pre-warmed SH-SY5Y medium without antibiotics, then split into 2 wells of a 24-well plate.
    iii. Incubate the cells for 2-3 days in the 37C/5% CO2 incubator.
    iv. After 2-3 days, one duplicate well is used for genomic DNA extraction and mismatch cleavage assay. The other sister well will be expanded depending on the KO efficiency analyzed by mismatch cleavage assay.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wei, J. (2023). Generation of SH-SY5Y HTT knockout cell lines by CRISPR-Cas9. Bio-protocol Preprint. bio-protocol.org/prep2366.
2. Bensalel, J., Xu, H., Lu, M. L., Capobianco, E. and Wei, J.(2021). RNA-seq analysis reveals significant transcriptome changes in huntingtin-null human neuroblastoma cells. BMC Medical Genomics 0(0). DOI: 10.1186/s12920-021-01022-w